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N-[^{18}F]-FluoropropylJDTic for κ-opioid receptor PET imaging: Radiosynthesis, pre-clinical evaluation, and metabolic investigation in comparison with parent JDTic

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ABSTRACT

Introduction: To image kappa opioid receptor (KOR) for preclinical studies, *N*-fluoropropylJDTic **9** derived from the best-established KOR antagonist JDTic, was labeled with fluorine-18.
Methods: Radiosynthesis of [^{18}F]**9** was achieved according to an automated two-step procedure from [^{18}F]-fluoride. Peripheral and cerebral distributions were determined by *ex vivo* experiments and by PET imaging in mouse. Radiometabolism studies were performed both *in vivo* in mice and *in vitro* in mouse and human liver microsomes. Identification of the major metabolic fragmentations was carried out by UPLC-MS analysis of enzymatic cleavage of non-radioactive ligand **9**. Microsomal metabolic degradation of parent JDTic was also achieved for comparison.
Results: The radiotracer [^{18}F]**9** was produced after 140 ± 5 min total synthesis time (2.2 ± 0.4% not decay corrected radiochemical yield) with a specific activity of 41–89 GBq/μmol (1.1–2.4 Ci/μmol). Peripheral and regional brain distributions of [^{18}F]**9** were consistent with known KOR locations but no significant specific binding in brain was shown. [^{18}F]**9** presented a typical hepatobiliary and renal elimination, and was rapidly metabolized. The *in vivo* and *in vitro* radiometabolic profiles of [^{18}F]**9** were similar. Piperidine **12** was identified as the major metabolic fragment of the non-radioactive ligand **9**. JDTic **7** was found to be much more stable than **9**.
Conclusion: Although the newly proposed radioligand [^{18}F]**9** was concluded to be not suitable for KOR PET imaging due to the formation of brain penetrating radiometabolites, our findings highlight the metabolic stability of JDTic and may help in the design of novel JDTic derivatives for *in vivo* applications.

1. Introduction

The kappa opioid receptor (KOR or κ) belongs to the superfamily of G-protein-coupled receptors and is specifically activated by endogenous opioids derived from prodynorphin [1–3]. KOR is widely distributed throughout the central and peripheral nervous systems, and has become an important molecular target for the study and the treatment of a variety of neuronal disorders such as anxiety, mood, pain, cognition, schizophrenia and addiction [4–12]. Preclinical studies have shown the potential clinical utility for KOR antagonists in depression and in alcohol, nicotine, cocaine and heroin dependence [13–21]. High expression of KOR has also been found in various cancer cells, such as the human adenocarcinoma breast cancer cell line MCF7 and small cell lung carcinoma [22–24]. As a result, an entry to preclinical and clinical PET imaging using selective radiotracers for mapping *in vivo* KOR and for probing their involvement in the psychopathologies and cancers is highly desirable.

Several compounds have been radiolabeled with carbon-11 [$t_{1/2}$ = 20.4 min, 99.8% β^+, E_{max} (β^+) 0.96 MeV] in order to develop specific PET probes for KOR imaging. The first ones were KOR agonists including U50,488 **1** [25], salvinorin A **2** [26,27], racemic GR89696 **3** [28] and its enantiomer GR103545 **4** [29,30] (Chart 1). Only [^{11}C]GR103545 ([^{11}C]**4**) was concluded to be a suitable KOR occupancy radiotracer and was evaluated extensively in nonhuman primates [30–32]. Although promising, the exploitation of [^{11}C]GR103545 in human was thought to be delicate. The administration of [^{11}C]GR103545, even at tracer doses, required a strict control to prevent the potential post-injection manifestation of KOR agonist effects such as dysphoria, sedation and psychosis [33]. Therefore, the radioligand has to be produced with very high specific activities in order to minimize the amount of injected product [34,35]. The newly reported [^{11}C]LY2795050 ([^{11}C]**5**) and [^{11}C]LY2459989 ([^{11}C]**6**) were the first KOR antagonist radiotracers evaluated in primate then in human [36–41] (Chart 1). These radioligands – that belong to the aminobenzyloxyarylamide class of compounds characterized by short-acting antagonistic effects *in vivo* and proven to have efficacy in the treatment of alcohol dependence and stress-related behaviors in rats [42,43] - were shown to display favorable brain uptake, pharmacokinetic properties and binding profile.

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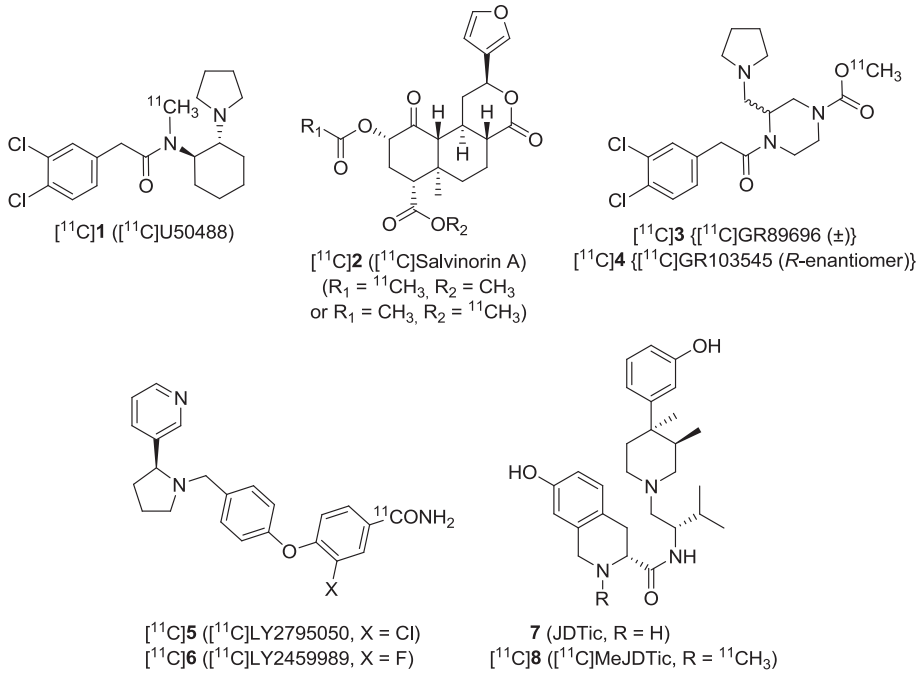

Chart 1. Chemical structures of KOR agonist and antagonist radioligands developed for PET imaging.

However, they may suffer from a lack of stability and KOR specificity [36,41]. [^{11}C]LY2795050 and [^{11}C]LY2459989 metabolized fairly quickly in plasma with parent fractions decreasing to 40% and 25% respectively at 30 min after injection in rhesus monkey or in human. Specific binding was only demonstrated in imaging by pretreatment with non-KOR-selective naloxone or naltrexone. *In vitro* pharmacological characteristics of the non-radioactive ligands revealed rather low KOR *versus* MOR (mu opioid receptor or μ) and DOR (delta opioid receptor or δ) selectivities in comparison with the best established pure selective KOR antagonist JDTic **7** [in assays using cloned human opioid receptors, for LY2795050 **5**: $Ki_{(\mu)}/Ki_{(\kappa)} = 35.8$, $Ki_{(\delta)}/Ki_{(\kappa)} = 212.5$; for LY2459989 **6**: $Ki_{(\mu)}/Ki_{(\kappa)} = 42.7$, $Ki_{(\delta)}/Ki_{(\kappa)} = 507$; for JDTic **7**: $Ki_{(\mu)}/Ki_{(\kappa)} = 191.6$, $Ki_{(\delta)}/Ki_{(\kappa)} = 3133$].

JDTic **7** possesses a trans-(3*R*,4*R*)-3,4-dimethyl-4-(3-hydroxyphenyl) piperidine structure known to provide pure opioid antagonist properties [44–48], and has become a useful tool for KOR structural features studies [49–52]. In binding assays, JDTic showed no detectable affinity (Ki ≥10 μM) for most non-opioid receptors and transporters present in the brain [48]. To date, JDTic remains the reference compound in the development of drugs for central nervous disorders [53–55]. JDTic was shown to display a robust effectiveness in various rodent models of depression, anxiety, alcohol seeking, nicotine withdrawal and stress-induced cocaine relapse. Contrary to aminobenzyloxyarylamide KOR antagonists, JDTic exhibited long-lasting pharmacokinetic properties. On the basis of the overall results, we considered that PET radioligands derived from JDTic **7** would represent an attractive alternative to [^{11}C]LY2795050 and [^{11}C]LY2459989. We previously developed *N*-[^{11}C]MeJDTic ([^{11}C]**8**) that exhibited a high specific binding to KOR in brain by *ex vivo* experiments in mouse [56] (Chart 1). As a continuation of our research efforts toward the development of JDTic-based radioligand for preclinical KOR PET imaging, we recently reported a series of fluoroalkyl derivatives [57]. Among them, *N*-fluoropropylJDTic **9** retained affinity for KOR and displayed selectivity *versus* MOR and KOR slightly higher than that of parent JDTic **7** [in assays with rat or human opioid receptors, for **9**: $Ki_{(\kappa)} = 1.6$ nM, $Ki_{(\mu)}/Ki_{(\kappa)} = 12$, $Ki_{(\delta)}/Ki_{(\kappa)} = 159$; for JDTic **7**: $Ki_{(\kappa)} = 0.42$ nM, $Ki_{(\mu)}/Ki_{(\kappa)} = 9$, $Ki_{(\delta)}/Ki_{(\kappa)} = 85$]. Modeling studies revealed that fluorine atom in **9** was involved in the specific KOR binding (Chart 2). In addition, the position of fluorine was appropriate for radiolabeling with fluorine-18, the radioisotope the most suitable for PET imaging ($t_{1/2} = 109.1$ min, 96.7% β^+, E_{max} (β^+) 0.63 MeV). Thus, the new ligand **9** was concluded to be a valuable candidate as radiotracer for KOR PET imaging. We reported here the radiosynthesis of the

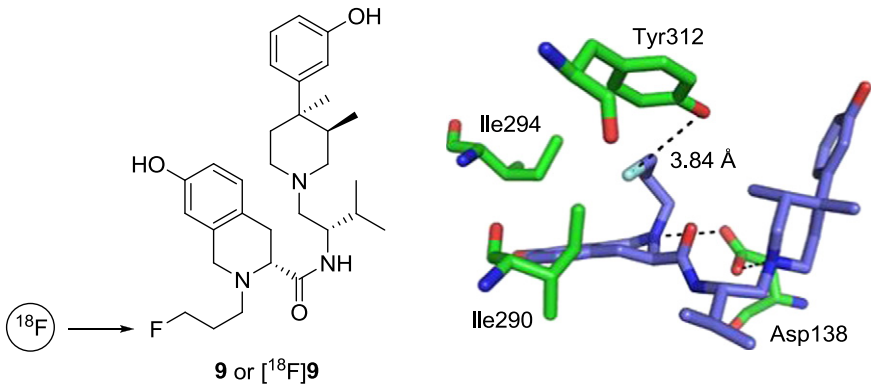

Chart 2. Chemical structure of *N*-fluoropropylJDTic **9** as candidate for radiolabeling with fluorine-18, and docking pose of **9** (purple carbon skeleton) in KOR binding pocket (green carbon skeleton). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)